## REMARKS

Reconsideration of this application is requested.

At the outset, the undersigned wishes to thank the Examiner (Mr. Rizzo) for kindly agreeing to conducting a personal interview on this application. The interview was held on July 25, 1995, and the courtesies extended by the Examiner were most appreciated.

The substance of the discussion will be evident from the following comments.

## I. FORMAL POINTS

It was noted at the outset that the Examiner has not acknowledged receipt of the certified priority documents for this application. Acknowledgement is requested in the next paper to issue in this case.

Claims 1 and 17 have been rejected as failing to comply with 35 USC 112, second paragraph, in view of the term "general". Claims 1 and 17 have been amended to delete "general". Reconsideration and withdrawal of that rejection is now respectfully requested.

## II. THE OBVIOUSNESS REJECTION

Claims 1-25 have been rejected under 35 USC 103 as being unpatentable over Wicha et al (Ar; hereinafter Wicha). That rejection is traversed for the following reasons.

It is known that certain enzymes are essential in the biosynthesis (in the body) of androgens and oestrogens. If these enzymes can be shut off, the

production of testosterone and the like can be shut off and control of prostatic cancer can be obtained. One of these enzymes is the hydroxylase-lyase enzyme. Tests can be done to evaluate the inhibition of individual steps catalyzed by this enzyme.

As explained during the interview, the present inventors have discovered that 17-pyridyl steroids are a class of hydroxylase-lyase inhibitors, and have narrowed this class further by showing that the relevant enzyme-blocking activities are possessed in significant measure only when the nitrogen atom of the pyridine ring is in the 3- or meta-position relative to the point of attachment of the pyridine ring to the steroid residue. Table 1 on page 5 of the specification shows the effect very dramatically. In that Table, enzyme activities are measured by $IC_{50}$ figures, $IC_{50}$ being the concentration of test compound required to achieve 50% enzyme inhibition. Thus, the lower the number in the column, the more effective is the test compound. It will be observed that the 3-pyridyl compound is roughly from 40 to 80 times more effective than the 2-pyridyl counterpart and 700 to 1200 times more effective than the 4-pyridyl counterpart. It is a highly critical feature of the invention, therefore that the 17-pyridyl group be a **17-(3-pyridyl)** group, i.e. one which has its pyridine nitrogen in the 3-position thereof.

Attention then turned to Table 2 on pages 37-38 of the specification which shows how the excellent properties of these steroids are attained with steroids having a variety of different structural features in their A, B and R rings. Clearly it is the D-ring which is important, independent of the nature of the A, B and C rings.

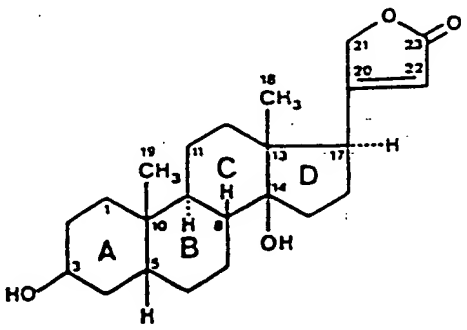
The compound claims are *per se* novel, except for a few which have been located in the literature and then only as **intermediates**. These are listed at page 2, line 17 - page 3 line 14. The literature concerned is the four Wicha papers AR, AS, At and AU cited by applicants. The compounds are excluded from the scope of claims to the compounds *per se*. All of these literature papers are concerned with

the synthesis of compounds alleged to have "cardiotonic" properties. A cardiotonic effect is one which stimulates the heart by increasing the force of myocardial contraction. It is also described as an inotropic effect on the heart.

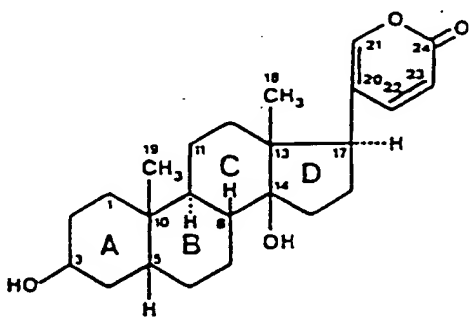
The compounds of the cited reference AR are analogous of the "cardiac glycosides". These heart-active steroids can be divided into two groups, the cardenolides and the bufadienolides. They all possess the normal steroid ring-system with a 14 beta-hydroxy- (or in certain rare cases a 14 beta, 15 beta-oxido-substituent), as well as an unsaturated lactone grouping in the 17 beta-position. The lactone consists of a butenolide (dihydro-oxofuryl) ring in the case of the cardenolides, and an alpha-pyrone ring in the case of the bufadienolides. The cardiac glycosides are active in the non-glycosidic form, usually referred to as "genins".

A typical member of the cardenolide group is digitoxigenin which occurs in the form of various glycosides in plants. Other cardenolides, all of them closely related in structure to digitoxigenin, occur as glycosides in the plant kingdom. Thus, digitoxigenin is the 16 beta-hydroxy-derivative of (A), digoxigenin is the 12 beta-hydroxy-derivative, while uzarigenin has the 5 alpha-digitoxigenin.

The bufadienolids, the second group of cardiac glycosides, are found in the poisonous secretion of toads. Bufalin is representative.




Digitoxigenin




Bufalin

It is helpful first to look at reference AT, because this paper explains that the researchers were looking for analogues of digitoxigenin and bufalin, replacing the unsaturated lactone ring substituents in the 17-position thereof, by a 17-(3-pyridyl) substituent. The only actual evidence that the compounds of the references AR, AS, AT and AU have cardiotonic such properties is reference AU, Wicha et al. Heterocycles 20, 231-234, at page 233, Table 1. These data are somewhat indeterminate, but indicate that the bufalin-like compound (2), with the unsaturated lactone ring, is **more** active than its pyridine analogue (1). This teaching therefore leads **away** from the idea of the 17-(3-pyridyl) substitution being a good one to make for cardiotonic purposes.

More importantly, both the "natural" cardiotonic steroids and their 3-pyridyl analogues all have saturated D-rings and 14-hydroxy or 14, 15-epoxy in compounds (10) and (11) of page 232 of AU. As noted during the interview, **the compounds of the invention do not possess these structural features.** Compound (9) of AU has a 14,15-double bond which is a permissible feature of the D-ring of the compounds of the present invention, but lacks the 16, 17-double bond required in the instant invention. Moreover, the compound is relatively **inactive** (see Table 1 of AU) and so is also contra-indicated.

This argument has been expanded and developed by a literature search performed for the specific purpose of responding to the outstanding action. Applicants have no interest in the cardiotonic field, so the references uncovered by this search are new. The following new references are cited:

| Ref Iden. | Citation |
|---|---|
| AAC | B.F. Hoffman, "The pharmacology of cardiac glycosides" in "Cardiac |

therapy", ed. M.R. Rosen and B.F. Hoffman, Martinus Nijhoff Publishers (1983) Chapter 11, pp. 387-412.

AAD R. Thomas et al. "Synthesis and Biological Activity of Semisynthetic Digitalis Analogs", J. Pharm. Sci. 63, 1649-1683 (1974).

AAE T. Shigei and S. Mineshita "Cardiotonic activities of four new compounds..." Experientia 24, 466-467 (1968).

AAF T. Shigei and S. Mineshita "Structure-activity Relationship of the Cardenolide..." Experientia 29, 449-450 (1973).

AAG M. Okada and Y. Saito "Synthesis of 3 beta-hydroxy-5 alpha-card-20...", Chem, Pharm. Bull. 16, 2223-2227 (1968).

AAH B.K. Naidoo et al. "Cardiotonic Steroids I: Importance of..." J. Pharm. Sci. 63, 1391-1394 (1974).

AAI W. Schonfield and K.R.H. Repke "A free-Wilson Analysis of 5 beta..." Quant. Struct. - Act. Relat. 7, 160-165 (1988).

AAJ T. Hashimoto et al. "Studies on Digitalis glycosides XXXV..." Chem. Pharm. Bull. 27, 2975-2979 (1979).

AAK K-O, Haustein et al "Structure-Activity relationships of natural and semi-synthetic..." Pharmacology 10, 65-75 (1973).

AAL Th. W. Guntert and H.H.A. Linde, "Cardiac glycosides: Prerequisites for the development..." Experientia, 33, 698-703 (1977).

**TABLE**

**Nomenclature 'primer' for cardiac glycosides**

| Name of compound | Steroid features (all have 14-OH and saturated D-ring) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 β | 3β | 4 | 5 | 10 | 11β | 12β | 16β | 17β |
| Digitoxigenin | - | OH | - | 5β-H | Me | - | - | - | Dihydro-oxofuryl |
| Uzarigenin | - | OH | | 5α-H | Me | - | - | - | " |
| Canarigenin | - | OH | $\Delta^4$ | - | Me | - | - | - | " |
| Digoxigenin | - | OH | - | 5β-H | Me | - | OH | - | " |
| Gitoxigenin | - | OH | - | 5β-H | Me | - | - | OH | " |
| Strophantidol | - | | | 5β-OH | $CH_2OH$ | - | - | - | " |
| Oubagenin | OH | OH | - | 5β-OH | $CH_2OH$ | OH | - | - | " |
| Bufalin | - | OH | - | 5β-H | Me | - | - | - | Pyrone |
| Scillarenin | - | OH | $\Delta^4$ | - | Me | - | - | - | " |

Referring to this new references, AAC and AAD are review papers cited for background information about the mechanism of the cardiac glycosides in order to enable the more specific papers to be better understood. The Hoffman reference, which is dated 1983, explains that the mechanism involves decreasing the activity of the "sodium-potassium pump" which is achieved by binding of the cardiac glycoside to a key enzyme in the operation of this pump - Na/L-AtPase. Cellular $Na^+$ levels increase, $Ca^{2+}$ levels increase, which in turn triggers further $Ca^{2+}$ to be released and cause cardiac contraction. It is for this reason that the putative cardiotonic compounds of Wicha et al were tested for inhibition of Na/K-ATPase. (Some years earlier, there were some doubts expressed about this mechanism, see AAD at page 1650, right-hand column and AAL. These seem to have been dispelled by the Hoffman review).

The other references fall into two groups. The first group, AAE to AAH, show that cardiotonic activity falls off when the 14β-hydroxy group present in the cardiac glycosides is replaced by a hydrogen atom. The second group, AAI-AAK, shows that cardiotonic activity falls off when a double bond is introduced between 16 and 17-carbon atoms of the D-ring.

These are **important** papers, because they show that no one wishing to improve the properties of cardiotonic compounds would make 14-desoxy derivatives (desoxy = hydroxy replaced by hydrogen) or $\Delta^{16,17}$ (16, 17 - unsaturated) derivatives. Thus, whatever may be the merits of replacing the dihydro-oxofuryl or pyronyl substituent in the 17-position by a 17-(3-pyridyl group if Wicha et al (AR, AS, AT, AU) were to be followed, **no one** skilled in the art would contemplate the further modifications of making a 14-desoxy or a $\Delta^{16,17}$ derivative, still less combining these two undesirable features, as happens in applicants' claimed compounds. They are combined in applicants' compounds because applicants are **not** concerned with the Na/K-ATPase enzyme or with cardiotonic properties; applicants are concerned with the 17α-hydroxylase/lyase enzyme and anti-cancer properties.

In the references, several different types of tests are used, yet the results are remarkably consistent. In AAE, 14-desoxyuzarigenin (VII) has a lower effect on myocardial contraction of a frog's heart than uzarigenin (VI) at two of the three concentrations for which comparative data are provided. The evidence in AAE is rather qualitative. It relates to 14-desocydigitoxigenin, which is rated as between 6 and 25 times less active than digitoxigenin in the frog's heart test. AAH contains tests on the inhibition of Na/K-ATPase and shows the inhibition constant $K_1$ increased (denoting less strong inhibition) from 6 x $10^{-8}$ in digitoxigenin to 1-6 x $10^{-6}$ in 14-desoxydigitoxigenin (VI), i.e. by more than 100-fold. This is highly consistent with AAG. Note in passing that the $\Delta^{14,15}$(= 14,15-ene) compound had an inhibition constant of 2.0 x $10^{-5}$, indicating it to be one of the poorest inhibitors. This is relevant as $\Delta^{14,15}$ compounds are within the scope of applicants' claim.

(Note: $K_1 = \frac{[\text{enzyme}]\,[\text{inhibitor}]}{[\text{enzyme-inhibitor complex}]}$

The greater the inhibition, the more the equilibrium will be towards the complex, the higher the complex concentration and the smaller the value of $K_1$).

AAF is cited only to confirm the structure of 14-desoxyuzarigenin referred to in AAE.

The second batch of specific references relates to the effect of 16,17-carbon unsaturation. In the present invention, this is **essential**, probably in order to allow the 3-pyridine ring, as the 17-substituent, to adopt the orientation required for coordination to the haem group of the hydroxylase-lyase enzyme. It will be appreciated that if the D-ring is saturated, i.e. as in the normal steroid structure, the 17-substituent could be axial (β) or equatorial (α). In fact, it is β in the cardiac glycosides, α having been shown to be unfavorable.

AAI is a paper in which inhibition of the Na/K-ATPase is measured. An inhibition or dissociation constant D is measured and converted into free energy using the well known relationship $\Delta G = RT \ln K_D$. (In this sign convention, the ΔG values are negative, so a large minus value of ΔG corresponds to a small value of $K_D$, which indicates tight binding of the inhibitor). Note initially that the well known genins digitoxigenin, digoxigenin, ditoxigenin and bufalin have large minus Δg values, consistent with their known good cardiotonic properties. The comparison of most interest is between compound 65, gitoxigenin, ΔG = 37.4 (Table 1) with compound 95, 16-anhydrogitoxigenin ΔG =-22.4 (Table 5). The latter compound is $\Delta^{16,17}$ (Table 6; also ref. AAJ, cited solely to confirm this nomenclature), i.e. it has a 16,17-double bond. The less negative ΔG value indicates that it is a weaker inhibitor. It is also to be noted that the diol 83 must have a 14-hydroxy substituent, even though this has been omitted from the name as printed. Otherwise, there would have been a mention of this departure from the normal structure in column 3 of Table 6).

It is also to be noted that introducing a 14,15-double bond or a 14,15β-epoxy group is unhelpful to cardiotonic properties. Compare digitoxigenin -3-acetate compound

20 where $\Delta G = 42.5$, with compound 91 which has the 14, 15-double bond in place of the 14-hydroxy group and has $\Delta G = 28.7$ and compound 93 which has the 14β, 15Δ-epoxide with $\Delta G = 36.4$.

Paper AAK records muscle contraction measurements on isolated guinea pig ileum. (The ileum is the bottom end of the smaller intestine which leads into the larger intestine). At first sight, the relevance of a paper which relates to the contraction of smooth muscle of the ileum might be questioned, but closer examination of the results for the principal compounds shows an interesting correlation with AAI.

| | ΔG per AAI | $ED_{50}$*per AAK |
|---|---|---|
| Gitoxigenin - 16-acetate | -43.4 | 0.054 |
| Digitoxigenin | -43.2 | 0.067 |
| Digitoxigenin -3-acetate | -42.5 | 0.104 |
| Gitoxigenin -3.16-diacetate | -39.9 | 0.13 |
| Oubagenin | -37.6 | 0.13 |
| Gitoxigenin | -37.4 | 0.46 |
| 16-Epigitoxigenin | -31.0 | 3.7 |

(*Presumably the concentration needed to produce half the maximal contraction. The lower the number, the more active the compound.)

The correlation is remarkable, considering that AAI relates to inhibition of the Na/K-ATPase and AAK to muscular contraction. In view of this striking correlation, AAK has been taken seriously as a predictor of cardiotonic efficacy. It is therefore relevant to observe that 16-anhydrogitoxigenin (the $\Delta^{16,17}$ unsaturated analogue) had an $ED_{50}$ of more than 30 in the guinea pig ileum test of AAK, i.e. about 75 times that of gitoxigenin, thus confirming AAI in which the respective free energies were -37.4 for gitoxigenin and -22.4 for the $\Delta^{16,17}$ unsaturated analogue.

The above discussion is believed to prove the point that a 14-hydroxy group and D-ring saturation are **essential** features for good cardiotonic activity. The references indicate

clearly to the person skilled in the art that compounds lacking these features would have considerably poorer cardiotonic properties, to the point where such compounds would **not** be obvious to prepare.

Since none of the cited art relates to the treatment of androgen- or estrogen-dependent disorders, it is believed that claims 17-24 are free of the prior art. Moreover, they are directed to non-obvious subject matter because the compounds in question have no medical use indicated whatever (cardiotonic use being unrealistic for the same reasons as for the compounds per se).

In addition to the above, it is clear that the remaining compounds of the instant invention (those not excluded by the disclaimer) are unobvious as intermediates in the preparation of cardiotonic compounds. Thus, reference AR describes an extraordinary long and complex synthesis of a compound (3) which is the same as the compound (1) of paper AU referred above. The detailed sequence of reactions is shown on the attached chart for ease of reference. The disclaimed compounds are (11), (12) and (13), of which (12) is a by-product obtained in 12% yield, (13) a by-product obtained in 6% yield and only (11) a proper intermediate (note: page 21 of AR, four lines beneath the formulae, is in error in referring to the "conjugated diene 18(12% yield)". It is in fact the conjugated diene 12: see page 24. Compound 18 is not a diene).

Considering compound (8) as the starting material, although this itself has to be synthesized in a yield of 80% or 87%, the ten subsequent steps to arrive at compound (3) result in overall yield of 6.0%. With a yield so low, a person of ordinary skill wishing to obtain compound (3) would not undertake any variations which might pass through compounds other than (11), absent some positive encouragement to do so. The Examiner has not cited another document giving such encouragement. Accordingly, the other compounds within claim 1 are not obvious as intermediates in the synthesis of cardiotonic steroids.

For all of the above reasons, it is clear that the claimed invention is not obvious over the art of record. There would have been no motivation to arrive at the present invention and, in the absence of any such motivation, it is clear that no *prima facie* case of structural obviousness has been made out. Withdrawal of the obviousness rejection on this ground alone is therefore in order and is requested.

Patentability of the claimed invention is further established by the attached Rule 132 Declaration by Dr. S. E. Barrie, one of the inventors. Dr. Barrie describes tests conducted on a compound within the scope of claim 1 and its D-ring saturated analogue, relating to inhibition of the 17α-hydroxylase-lyase enzyme relevant to the instant invention. Compound (1) in the Table in paragraph 7 is a compound of the invention, compound (2) is its D-ring-saturated analogue. The fall off in inhibitory activity with D-ring saturation is in the region of 10-fold. A comparison is also shown for the corresponding 17-(4-pyridyl) compounds (3) and (4) where the reverse trend was seen, but the 4-pyridyl compounds were relatively inactive.

Dr. Barrie's declaration establishes the criticality of the 16, 17-double bond for the purpose of inhibition of the 17α-hydroxylase-lyase enzyme relevant to the instant invention. It is believed unnecessary to rely on Dr. Barrier's declaration, since D-ring saturated compounds are not being claimed, since the only objection to the claims is based on cardiotonic properties and since D-ring-unsaturation is contra-indicated by the prior art relating to compounds having such properties. Nevertheless, it is interesting that the very structural feature which is contra-indicated by the prior art is also essential for the purposes of the present invention.

During the interview, the Examiner suggested amending the claims to be commensurate in scope with the evidence. The applicants have carefully reviewed the claim scope in light of the evidence and have concluded that the claims properly focus on the inventive concept underlying this invention. Thus, the claims focus on the compounds of formula (1) containing a 17-(3-pyridyl) substituent and unsaturation at the 16-position of

the D-ring. The criticality of this structural feature is established by the evidence of record, and further limitation elsewhere in the structure is clearly unnecessary.

Applicants' copy of the first PTO 1449 initialed by the Examiner does not include the second sheet listing references AT to AAA. Also the second PTO 1449 listing reference AAB was not returned duly initialed. Rectification of this mailing error would be sincerely appreciated. In addition, the Examiner is requested to kindly initial and return the third PTO 1449 provided herewith.

Allowance of the application is respectfully requested.

Respectfully submitted,

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